Risk factors for antibiotic resistance in *Streptococcus pneumoniae*

Keith P Klugman

Streptococcus pneumoniae (the pneumococcus) is the leading vaccine-preventable cause of death in children and adults. The management of pneumococcal infections is complicated by the development of resistance to antimicrobials. Risk factors for increased resistance include young age, isolation from the upper respiratory tract, hospitalisation, residence in an urban area, day care attendance, previous exposure to antibiotics, female gender, exposure to specific serotypes and clones, HIV infection and exposure to a class of drug to which resistance can be easily selected from a susceptible population of organisms. Conjugate pneumococcal vaccine affords protection from resistant strains belonging to vaccine serotypes, but resistance is emerging in non-vaccine types.

Acute respiratory infections are the leading infectious cause of death in both adults and children.[1] The main pathogen responsible for this mortality is the pneumococcus, *Streptococcus pneumoniae*, and its treatment is complicated by the evolution of resistance to antibiotics. The first description of fully penicillin-resistant and multiply-resistant pneumococci was made by Professor Hendrik Koornhof and his colleagues at Baragwanath Hospital, Soweto, South Africa and reported in the *New England Journal of Medicine* in 1978.[2] These multiply-resistant strains were nosocomial in origin and were restricted initially to infections among hospitalised children. Over the intervening 25 years, multiply-resistant strains developed a global distribution[3] and there is almost no place on earth where the management of pneumococcal infections in both children and adults does not need to take into account the possibility of antimicrobial resistance. This review of the factors underlying that global expansion of resistant strains is dedicated to Hendrik Koornhof, my mentor, and the mentor of a generation of South African clinical microbiologists.

Age

Pneumococci resistant to beta-lactams and macrolides occur more frequently among children than adults,[4-6] reflecting both the prolonged duration of carriage of pneumococci in children compared with adults[7] and the greater per capita use of antibiotics in children compared with adults. The exception to the association of young age with resistance in the pneumococcus is resistance to fluoroquinolones. This class of agent is not licensed for use in children, except for exceptional circumstances. Given that the burden of use to treat pneumococcal infections is in elderly adults, it is not surprising that this resistance emerged in the elderly[8] and is associated with nosocomial exposures to fluoroquinolones in older patients with exacerbations of chronic bronchitis.[9] Fluoroquinolone-resistant pneumococci are also more common in elderly persons living in long-term care facilities.[10]

Site of specimen

Resistance is more common in isolates from the respiratory tract,[4] rather than from invasive disease specimens such as blood or cerebrospinal fluid (CSF). This reflects both the likely selection of resistant strains in the nasopharynx, where there is a high density of organisms and antibiotic concentrations tend to be low, and a bias towards the collection of respiratory specimens only after initial treatments have failed. Respiratory specimens therefore mainly follow previous antibiotic therapy. A similar bias may allow resistant strains to be found more often in blood than CSF,[5] as previous exposure to oral antibiotics may clear susceptible strains from blood, thus increasing the proportion of resistant strains, whereas previous antibiotics are less likely to eradicate susceptible strains from the CSF.

Hospitalisation

Nosocomial acquisition is an important risk for the selection of resistant pneumococci,[2,4,9] as is exposure to a nursing home.[9,10]

Urban versus rural

The carriage of resistant strains was compared between a cluster randomised sample of children in rural Lesotho villages, some accessible only by donkey, and similarly aged children from Maseru, the capital.[11] Both penicillin and tetracycline resistance were significantly higher in urban versus rural children, probably due to significantly increased exposure of the urban children to recent antibiotic use (OR 8.8, $p<0.01$); past hospitalisation (OR 24.8, $p<0.001$); and more day care attendance (OR 13.1, $p<0.001$). These factors outweighed crowding, which was less common in the urban setting with significantly less children <5 years of age in the household (OR 0.4, $p<0.001$).[11]

William H Foege Professor of Global Health, Rollins School of Public Health, Emory University, Atlanta, USA; Professor of Clinical Microbiology, MRC/NICD/Wits Respiratory and Meningeal Pathogens Research Unit, Johannesburg
Keith P Klugman, MB BCh, PhD, FRSSAf

Corresponding author: *K Klugman (keith.klugman@emory.edu)*

Day care

Day care is a significant risk for selection of resistant strains in both developing[11] and developed[12] country settings due to crowding and lack of hygiene that facilitate spread, as well as increased exposure to antibiotics which facilitate selection of resistant strains. Multiresistant pneumococcal clones have been shown to spread more quickly and to colonise more children in day care than do susceptible pneumococcal clones.[13]

Antibiotic use

By far the most important risk for antimicrobial resistance in the pneumococcus is exposure to antibiotic use. This has been demonstrated at the country level where there is a clear association of pneumococcal resistance with antibiotic use in the countries of the European Union;[14] at county level where resistance has been correlated with use within counties in Iceland;[15] and at the level of the individual where resistance associated with individual exposure has been known since the early 1980s.[16] The duration of increased risk has been demonstrated to be similar to the duration of carriage of pneumococci, i.e. 2 - 7 weeks.[15] The relationship between antimicrobial resistance and exposure is complicated by the emergence of multiply-resistant pneumococci that may be selected by several classes of antibiotics. Modelling suggests that strains resistant to multiple classes of antibiotics have spread at the expense of both susceptible strains and those resistant only to one class[17] and it has been shown that certain drugs, such as azithromycin, which has a long half-life, may be more important in the selection of multiply-resistant strains than may drugs from other classes of antimicrobial agent.[18] Not only do antibiotics of various classes select for resistance, but drugs directed against similar targets in unrelated species, such as the malaria parasite, may lead to resistance in the pneumococcus. Fansidar, which acts to inhibit folate synthesis in falciparum, may select for pneumococci resistant to folate antagonists, and such selection has been documented among children receiving the drug in Malawi.[19] Duration of use and dose as risks for resistance in the pneumococcus have been examined in only two studies to date, one retrospective[20] and one prospective,[21] both concluding that short duration and higher dose select less for resistance than do longer durations of exposure and lower doses. Although both appropriate and inappropriate antibiotic use may select for resistance, inappropriate use (e.g. to treat upper respiratory viral infections) may exceed appropriate use in many settings and the large differences in pneumococcal resistance found in Germany (less resistance) versus France (more resistance) have been suggested to reflect, at least in part, the frequency with which inappropriate antibiotic use for respiratory indications occurs in France[22] compared with Germany.

Gender

Although pneumococcal disease has a male predominance among adults in Soweto, it has been shown that females have more resistant pneumococcal infections than men,[23] probably related to their increased exposure to resistant strains transmitted from children.

Serotype

The so-called 'paediatric serotypes' of pneumococci are the strains commonly found in children, and these strains are carried in the nasopharynx for longer times than other serotypes.[7] These types are more resistant to antibiotics than are types more often found in adults, which are less often to be found in carriage.[24,25]

Clones

Within the capsular types of pneumococci there are a number of antibiotic-resistant pneumococcal clones that have spread globally.[26] These strains have over the past 29 years become the dominant pneumococci isolated from invasive infections in most developed countries and are documented by a collaboration of scientists called the Pneumococcal Molecular Epidemiology Network (PMEN) (http://www.sph.emory.edu/PMEN/). The preponderance of these global clones can be considerable, and an analysis of the clonality of invasive pneumococci fully resistant to penicillin, before the introduction of pneumococcal conjugate vaccine in the USA, showed that 93% of these invasive strains belonged to just eight clones, of which five were PMEN global clones.[27]

HIV

The antibiotic-resistant pneumococcus is more commonly found among both children and adults infected with HIV than among those who are HIV-uninfected.[23,28] This reflects a number of risk factors including increased hospitalisation, increased infections with paediatric pneumococcal serogroups and increased exposure to antibiotics, not only therapeutically, but also prophylactically[29] among HIV-infected people.

Mechanism of resistance – ease of selection

A review of mechanisms of resistance to antimicrobials is beyond the scope of this article, but certain mechanisms may facilitate selection of resistance above others. Resistance to beta-lactam antibiotics requires multiple step-wise mutations in the genes encoding penicillin-binding proteins; resistance to macrolides is usually due to acquisition of exogenous efflux or methylating enzymes, but only rarely due to mutations in ribosomal proteins or ribosomal RNA; and resistance to

fluoroquinolones requires at least two step-wise mutations in the topo-isomerase genes. Once the resistance genes are acquired, antibiotic use may select resistant strains, but ease of initial selection from a susceptible population depends on the frequency with which resistant strains can arise from the susceptible population. Resistance to the classes mentioned above rarely arises from susceptible strains and dissemination requires pre-existing resistant strains. Pre-existing first-step fluoroquinolone mutants may therefore be a significant risk for the evolution of subsequent fully resistant strains in long-term care facilities.[30] The most common resistance determinant in the pneumococcus globally, however, is resistance to trimethoprim-sulphamethoxazole (TMP-SMZ), and this resistance can be selected with a high frequency (in >30% of exposed individuals after a week of exposure).[19] A factor in the frequency of the selection of this resistance is that it is conferred by just a single nucleotide base change in the gene encoding dihydrofolate reductase,[31] and can thus be selected with ease from susceptible populations of $>10^7$ pneumococci. Although TMP-SMZ is less often used these days to treat pneumococcal infections, its widespread use as prophylaxis in HIV-infected children and adults represents a significant risk for selection of resistance. Of particular concern is the observation that TMP-SMZ use was the most significant risk for penicillin-resistant pneumococci in an African setting in which strains resistant to both drugs were common.[32]

Vaccine impact

In contrast to the factors above that all contribute to an increase in resistance, the introduction of conjugate pneumococcal vaccine in children, directed against the paediatric serotypes that included most of the serotypes associated with resistance, has been, at least initially, associated with a reduction in resistance. Pneumococcal polysaccharide conjugated to diphtheria cross-reacting molecule (CRM) prevents the acquisition of antibiotic-resistant strains belonging to vaccine serotypes[33-35] and has been shown to reduce resistant infections among vaccinated children in a prospective randomised trial.[36] Effectiveness studies in the USA demonstrated the reduction in antibiotic resistance in invasive pneumococcal infections after introduction of conjugate vaccine, not only in children but also in adults due to herd immunity.[37-39] Unfortunately, in isolates from the respiratory tract it has quickly become evident that continuing antibiotic use is selecting resistance in non-vaccine isolates.[40] This meant that little impact on resistance was found in nasopharyngeal isolates[41,42] or ear isolates[43] after vaccination. Recently there has been a significant expansion of antibiotic-resistant invasive serotype 19A infections after vaccine licensure in the USA.[44-47] It is clear therefore that continuing exposure to antibiotics in the post-vaccine era will result in expansion of resistance among non-vaccine serotypes. The relative contributions of vaccination to reduce resistance, and antibiotic to increase resistance, have been studied in children attending day care in France. Children at lowest risk of resistant strains (vaccinated and with no recent antibiotic exposure) had only 4% carriage of penicillin-resistant pneumococci, compared with 16% carriage of resistant strains among those at high risk (not vaccinated and recently received antibiotics).[48]

Conclusions

Multiple risks exist for the selection and dissemination of antibiotic-resistant pneumococci. These risks have led to the global dissemination of multiply-resistant pneumococcal clones, particularly, but not exclusively among children. Most of the risk factors for resistance have a common thread, namely exposure to the antibiotic to which the pneumococcus is resistant. Conjugate pneumococcal vaccine dramatically reduced resistance among invasive pneumococci after its introduction, but resistance is emerging in non-vaccine types and the continued selective advantage of resistance in the presence of antibiotic may overwhelm the vaccine impact on resistance in the future.

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